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Analysis of glimepiride by using derivative UV spectrophotometric method

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Abstract

Glimepiride (Amaryl®), which is a new oral antidiabetic drug in the sulfonylurea class, was analysed by using second order derivative UV spectrophotometry. The quantification of glimepiride in dimethylformamide was performed in the wavelength range of 245–290 nm at $N=6$, $?\lambda=21$. The second order derivative spectra was calculated using peak to peak ($\lambda_{DMF}=263.3$–268.2 nm), peak to zero ($\lambda_{DMF}=268.2$ nm) and tangent ($\lambda_{DMF}=263.3$–271.8 nm) method for calibration curves, the linearity range of 1.00–500.00 μg ml^{-1} by using the second order derivative UV spectrophotometric method. The developed method was applied to directly and easily to the analysis of the pharmaceutical tablet preparations. R.S.D. were found to be 4.18% (Amaryl® tablet; 1 mg) and 2.21% (Amaryl® tablet; 2 mg). The method was completely validated and proven to be rugged. The limit of quantitation and the limit of detection were found as 1.00 and 0.4 μg ml^{-1}, respectively. This validated derivative UV spectrophotometric method is potentially useful for a routine laboratory because of its simplicity, rapidity, sensitivity, precision and accuracy. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Glimepiride; Derivative UV spectrophotometry; Pharmaceutical dosage form (tablet)

1. Introduction

The chemical formula of glimepiride is 1-H-pyroll-1-carboxamide-3-ethyl-2,5-dihidro-4-methyl-*N*-[2-[4-[[[[(4-methylsiklohexyl)amino]carbonyl]amino]sulfonyl]phenyl]ethyl]-2-oxo-*trans* (Fig. 1).

Glimepiride is a new oral antidiabetic drug in the sulfonylurea class having a prolonged effect [1]. In order to achieve appropriate control of blood glucose level, the treatment of non-insulin dependent Type II diabetes usually starts with diet and exercise. If this still results in insufficient metabolic control, oral hypoglycemic drugs or insulin added to the non-pharmacological measures [2,3]. Glimepiride achieved metabolic control with the lowest dose (1–8 mg daily) of all the sulphonylureas. In addition, it maintains a more physiological regulation of insulin secretion than glibenclamide during physical exercise, suggesting that there may be less risk of hypoglycaemia with glimepiride [4–6].

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The pharmacokinetic profile of glimepiride has been assessed in healthy volunteers and non-insulin dependent diabetes mellitus (NIDDM) patients after oral administration [7–9].

Glimepiride is only analysed by high performance liquid chromatographic (HPLC) method in biologic material [10] and no derivative spectrophotometric method in any material has been reported in the literature.

The proposed method cheaper and more simple than HPLC method.It might be an alternative to the HPLC techniques for routine analysis and there are no extraction proceses to eliminate the excipients, which are time concuming and tedious.

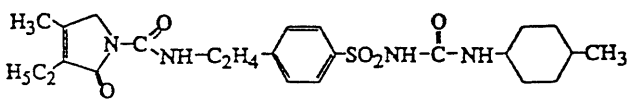

Fig. 1. Chemical structure of glimepiride.

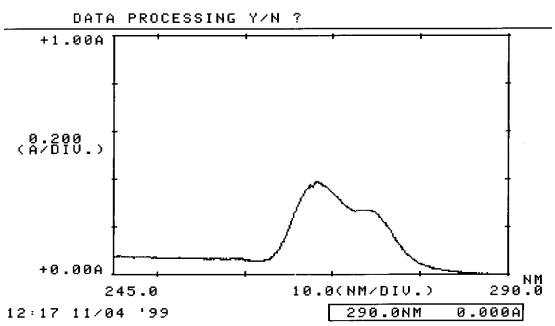

Fig. 2. The original UV spectrum (zero order derivative) of glimepiride (200 μg ml^{-1} glimepiride in DMF).

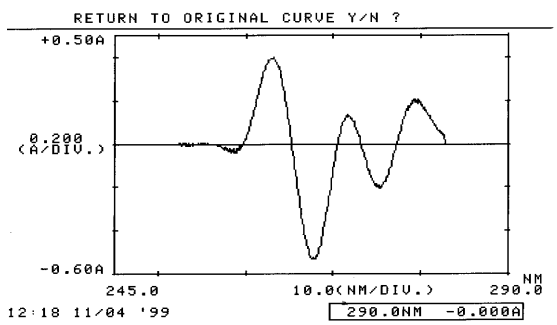

Fig. 3. Second order derivative spectrum of glimepiride (200 μg ml^{-1} glimepiride in DMF).

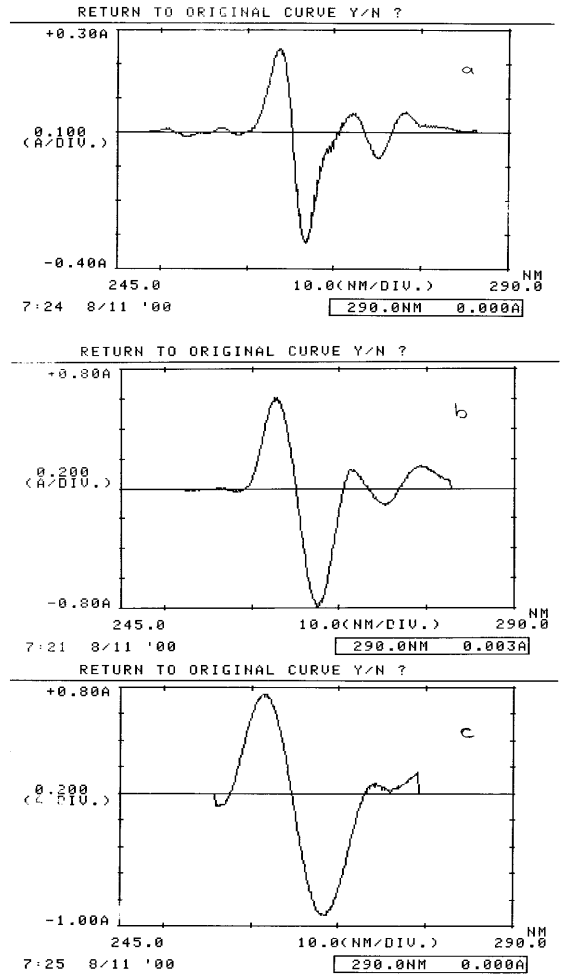

Fig. 4. Second derivative spectrum of glimepiride (200 μg ml^{-1} glimepiride in DMF). (a) $N=3$, $\Delta\lambda=10.5$; (b) $N=6$, $\Delta\lambda=21.5$; (c) $N=9$, $\Delta\lambda=31.5$.

The aim of this study is to develop a simple, sensitive and validated, derivative UV spectrophotometric method for the determination of glimepiride and to apply this method to the commercial pharmaceutical tablet preparations.

2. Experimental

2.1. Instrument

A Shimadzu UV-160 recording double beam UV–visible spectrophotometer with data process-

ing system was used. UV spectra of reference and sample solutions were recorded in 1-cm quartz cells at a scan speed of 50 nm min^{-1} with a fixed slit width of 3 nm. The concentration of glimepiride in its solutions in dimethylformamide (DMF) were determined between in wavelength ranges of 245–290 nm ($N = 6$; $\Delta\lambda = 21.0$).

2.2. Reagents and solutions

The glimepiride standard was donated by the Hoechst Marion Roussel drug company. Purity of the glimepiride was tested by checking its melting point, UV and IR spectra and no impurities were found. All analytical chemicals were purchased from Merck. Stock solution of glimepiride (1000 μg ml^{-1}) was prepared in DMF. Working standard solution was prepared by diluting the stock solution in the concentration range from 1.0 to 500.0 μg ml^{-1} with DMF daily.

2.3. Procedure

Ten tablets of glimepiride were totally weighed and powdered. An amount of this powder corresponding to one tablet glimepiride content was weighed in to a 10-ml volumetric flask, 5 ml DMF was added and the flask was sonicated for 5 min. The flask was filled to volume with DMF. Appropriate dilutions were done into the range of calibration curve with DMF. The second order derivative UV spectra of the resulting solutions were recorded against DMF as a reference solution.

Table 1
The results of calibration curve with three methods measured in DMF ($n = 11$)[a]

Method	Calibration curve	r	S.E. of slope	S.E. of intercept
Peak to peak	$y = 0.0031x + 0.1025$	0.9998	2.94×10^{-3}	3.46×10^{-3}
Peak to zero	$y = 0.0018x + 0.0556$	0.9996	8.64×10^{-4}	5.37×10^{-4}
Tangent	$y = 0.0024x + 0.0696$	0.9997	7.51×10^{-3}	4.37×10^{-4}

[a] r, the coefficient of correlation; x, concentration of glimepiride; y, the amplitude of second order derivative spectrum.

Table 2
Specificity results of the second order derivative UV spectrophotometric method[a]

Pure sample		Sample spiked with all impurities	
1 mg	2 mg	1 mg	2 mg
1.05	2.04	1.03	2.02
1.03	2.06	0.96	1.97
0.98	2.02	1.03	2.12
0.96	2.05	0.92	2.08
1.07	1.96	0.97	2.06
1.02	2.01	1.01	2.05
1.09	2.12	1.04	1.96
$\bar{X} = 1.028 \pm 0.016$	$\bar{X} = 2.037 \pm 0.017$	$\bar{X} = 0.994 \pm 0.02$	$\bar{X} = 2.037 \pm 0.02$
S.D. = 0.043	S.D. = 0.045	S.D. = 0.042	S.D. = 0.054
R.S.D. = 4.18%	R.S.D. = 2.21%	R.S.D. = 4.22%	R.S.D. = 2.65%
C.I. = 0.989–1.067	C.I. = 1.995–2.079	C.I. = 0.955–1.032	C.I. = 1.988–2.086

[a] Results are mean seven separate measurements of peak to peak method.

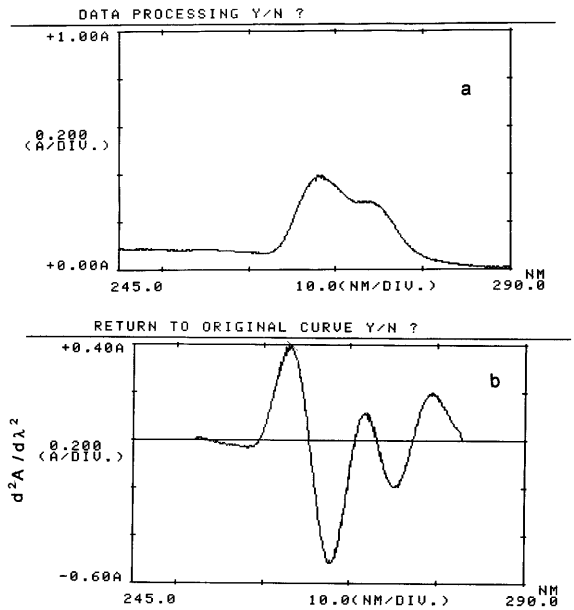

Fig. 5. (a) Zero order spectrum; (b) second order derivative spectrum of glimepiride in pharmaceutical preparations.

3. Results and discussion

3.1. Method development

The solvent, the degree of derivation, the wavelength range and N values were chosen in order to optimise the conditions. Glimepiride is not soluble in water, acid, base, borate and phosphate buffers but partly soluble in methanol, ethanol, acetone and ethylacetate, but completely soluble in DMF [11]. Thus DMF was chosen as solvent for preparation of glimepiride solution. UV spectrum of glimepiride in DMF gave two broad shouldered peaks with maximum wavelengths at 268.2 and 271.8 nm, respectively (Fig. 2). These maximum wavelengths were broader at low concentrations so that analysis couldn't be performed; at higher concentrations the peaks were sharper but analysis couldn't be carried out because of the shoulder.

Table 4
The results of percentage recovery value 200 μg ml^{-1} reference standard solutions in DMF by the developed second order derivative UV spectrophotometric method[a]

Found glimepiride (μg ml^{-1})	Recovery (%)
200.1	100.05
200.5	100.25
199.8	99.90
200.3	100.15
199.6	99.80
200.7	100.35
200.4	100.20

[a] Results are mean seven separate measurements of peak to peak method. $\bar{X} = 200.08 \pm 0.13$; S.D. = 0.362; R.S.D. = 0.18%; mean recovery = 100.10%.

Table 3
The results of analysis of pharmaceutical preparations containing glimepiride by second order derivative UV spectrophotometric method[a]

Amaryl® tablet (1 mg glimepiride)		Amaryl® tablet (2 mg glimepiride)	
Calibration curve method	Standard addition method	Calibration curve method	Standard addition method
1.05	1.07	2.04	2.07
1.03	1.05	2.06	2.11
0.98	1.01	2.02	1.94
0.96	0.98	2.05	2.08
1.07	1.09	1.96	2.01
1.02	1.03	2.01	2.04
1.09	1.11	2.12	2.13
$\bar{X} = 1.028 \pm 0.016$	$\bar{X} = 1.048 \pm 0.015$	$\bar{X} = 2.037 \pm 0.017$	$\bar{X} = 2.054 \pm 0.022$
S.D. = 0.043	S.D. = 0.042	S.D. = 0.045	S.D. = 0.059
R.S.D. = 4.18%	R.S.D. = 4.01%	R.S.D. = 2.21%	R.S.D. = 2.87%
CI = 0.989–1.067	CI = 1.011–1.085	CI = 1.995–2.079	CI = 2.000–2.108

[a] Results are mean seven separate measurements of peak to peak method.

Derivative UV spectrophotometry was prefered for the analysis of glimepiride since the amplitude of the signal of derivative spectra was greater, the peak shape was well defined and the separation of the shouldered peaks was better in this method.

The second order derivative UV spectrum analysis of glimepiride gave sharper and better-defined peaks when compared with the zero order derivative spectrum of glimepiride (original) (Fig. 3).

The derivative wavelength difference ($\Delta\lambda$) depends on the measuring wavelength range and the key entry N (smoothing factor) optimal wavelength range should be chosen since the broad peaks get sharper, the ratio of signal/noise (S/N) elevates and the sensitivity of the method, increases by controlling same degree of low-pass filtering or smoothing. Therefore, a series of N value ($N = 1–9$) were tested by second order UV spectrum of glimepiride in DMF (Fig. 4). The optimum N value was found to be $N = 6$ ($\Delta\lambda = 21$) in the measuring wavelength range of 245–290 nm.

Table 5
The results of percentage recovery value in synthetic mixture of glimepiride by the developed second order derivative UV spectrophotometric method (added glimepiride for tablet 1; mg)[a]

Calibration curve found glimepiride (mg)	Recovery (%)	Standard addition method found glimepiride (mg)	Recovery (%)
1.03	103.00	1.07	107.00
0.96	96.00	1.04	104.00
1.03	103.00	0.97	97.00
0.92	92.00	1.05	105.00
0.97	97.00	0.96	96.00
1.01	101.00	1.03	103.00
1.04	104.00	1.02	102.00
$\bar{X} = 0.994 \pm 0.02$	$\bar{X} = 99.71 \pm 1.6$	$\bar{X} = 1.02 \pm 0.014$	$\bar{X} = 102 \pm 1.4$
S.D. = 0.042	S.D. = 4.16	S.D. = 0.038	S.D. = 3.78
R.S.D. = 4.22%	R.S.D. = 4.17%	R.S.D. = 3.72%	R.S.D. = 3.70%

[a] Results are mean seven separate measurements of peak to peak method.

Table 6
The results of percentage recovery value in synthetic mixture of glimepiride by the developed second order derivative UV spectrophotometric method (added glimepiride for tablet; 2 mg)[a]

Calibration curve found glimepiride (mg)	Recovery (%)	Standard addition method found glimepiride (mg)	Recovery (%)
2.02	101.00	2.05	102.50
1.97	98.50	2.01	100.50
2.12	106.00	2.15	107.50
2.08	104.00	2.11	105.50
2.06	103.00	2.08	104.00
2.05	102.50	2.07	103.50
1.96	98.00	2.02	101.00
$\bar{X} = 2.037 \pm 0.02$	$\bar{X} = 101.86 \pm 1.01$	$\bar{X} = 2.07 \pm 0.02$	$\bar{X} = 103.5 \pm 0.87$
S.D. = 0.054	S.D. = 2.68	S.D. = 0.046	S.D. = 2.28
R.S.D. = 2.65%	R.S.D. = 2.63%	R.S.D. = 2.22%	R.S.D. = 2.20%

[a] Results are mean seven separate measurements of peak to peak method.

Table 7
Intraday and interday precision and accuracy of glimepiride

Intraday[a]					Interday[b]			
Added concentration (μg ml^{-1})	Measurement concentration (mean) (μg ml^{-1})	Precision		Accuracy[c] (%)	Measurement concentration (mean) (μg ml^{-1})	Precision		Accuracy[c] (%)
		S.D.	R.S.D.(%)			S.D.	R.S.D. (%)	
50	50.4	0.72	1.42	100.8	50.8	1.05	2.06	101.6
100	100.6	0.84	0.83	100.6	100.7	1.13	1.12	100.7
200	200.3	0.79	0.39	100.2	200.4	1.18	0.58	100.2
400	400.2	0.81	0.20	100.1	400.3	1.09	0.27	100.1

[a] Mean values represent seven different glimepiride standard for each concentration.
[b] Interday was determined from seven different runs over a 4-week period. The concentration of each run was determined from a single calibration curve run on the first day of the study.
[c] Accuracy = (observed concentration/theoretical concentration) × 100.

3.2. Method validation

3.2.1. Linearity range

In quantitative analysis of glimepiride the calibration curves were plotted using second derivative spectra in DMF. Linearity was checked by preparing standard solutions at 11 different concentrations, ranges from 1.00 to 500.00 μg ml^{-1}. The second order derivative spectrum was evaluated by using peak to peak, peak to zero and tangent methods. The results of calibration curves with three methods measured in DMF were given Table 1.

These results show that three derivative spectrum evaluation methods can be used. The slope of the peak to peak calibration curve in DMF was higher than the others and this method was used in the rest of study for calculations. The linearity ranges were found to be 1.00–500.00 μg ml^{-1} in DMF by using the values obtained from the second order derivative UV spectrum of the substance. Peak to peak was measured between wavelengths 263.3–268.2 nm in DMF.

3.2.2. Sensitivity

The limit of quantitation (LOQ) of glimepiride was 1.00 μg ml^{-1} in DMF. The limit of detection (LOD) was found as 0.4 μg ml^{-1} in DMF. LOD was considered as the glimepiride concentration having a S/N ratio greater than 2.81.

3.2.3. Specificity/selectivity

Comparison of the original and second order derivative spectrum of glimepiride in standard and drug formulation solutions show that the wavelength of maximum absorbance did not change. Specificity is the ability of the method to measure the analyte response in the presence of all the potential impurities. For the specificity test, all known impurities were added to the pure glimepiride sample and response of the analyte in this mixture was compared with the response of pure glimepiride. It was found that assay results were not changed in the presence of the impurities. The assay results were given in the Table 2. Therefore, impurities did not interfere the quantitation of glimepiride in tablet. Second order derivative UV spectrophotometry presents and advantage over spectrophotometry in tablet formulations, because pharmaceutical preparations yielded turbid solutions. In the proposed method there was no need pre-separation and only centrifugation was applied to make the solution clear.

3.2.4. Application of method to the pharmaceutical preparations

The developed second order derivative UV spectrophotometric method was applied to the tablet formulations, zero order and second order UV spectrum of glimepiride in tablet formulation were given in Fig. 5. The results of calibration curve and standard addition technique for glimepiride quantitation in tablets (Amaryl® tablet; 1 mg and Amaryl® tablet; 2 mg) were also given in Table 3.

3.2.5. Accuracy

Standard addition and recovery experiments were conducted to determine the accuracy of the proposed method. In order to detect interactions of the excipients in this method, the standard addition technique was applied to the same preparations that were analysed by the calibration curve. The regression equation of standard addition curve was found as $y = 0.0032x + 0.3199$ ($r = 0.9998$) where y is the amplitude of second

Table 8
The result of analyses from pharmaceutical preparations and standard of glimepiride by two different analysts and instruments[a]

	Different analyst			Different instrument		
	$\bar{X}$	S.D.	R.S.D. (%)	$\bar{X}$	S.D.	R.S.D. (%)
Standard of glimepiride (100 μg ml^{-1})	100.4	0.84	0.83	100.5	0.94	0.93
Tablet (1 mg glimepiride)	1.01	0.04	3.96	1.02	0.05	4.90

[a] Results are mean seven separate measurements of peak to peak method.

derivative spectrum, x is concentration of glimepiride and r is the coefficient of correlation. Since the slopes of the standard and standard addition curves were identical (Table 1). It was concluded that there was no spectral interaction in the analysis of pharmaceutical preparations. There was no difference between the R.S.D. of the two techniques (Table 3)

Recovery studies were performed at a concentration of 200 μg ml^{-1} glimepiride standard solutions in DMF ($n=7$).The mean recovery and R.S.D. were found to be 100.10 and 0.18%, respectively (Table 4).

The other recovery study was performed on the synthetic mixture prepared by adding accurately weighed amounts of glimepiride to the excipient mixture (corn starch, magnesium stearate, lactose, talc, the colour coating contains red ferrioxit (1 mg glimepirid) and yellow ferrioxit (2 mg glimepiride)) by calculating the percentage of recovery in mean $\pm$ S.D. ($n=7$) in each case (Tables 5 and 6).

3.2.6. Precision

To determine the precision of the method, glimepiride solutions at a concentration of 200 μg ml^{-1} in DMF were analysed seven times and the mean glimepiride value were found as 200.03 μg ml^{-1}. The S.D. was found as 0.79. Suggesting that the developed method has a good precision.

Repeatability is given as interday and intraday precision and accuracy where evaluated by analysing four different concentration of glimepiride. The results are given Table 7 [12,13].

3.2.7. Robustness and ruggedness

The ruggedness test of analytical assay method is defined as degree of reproducibility of assay results obtained by the successful applications of the assay over time and among multiple laboratories and analyst [12]. The robustness of presented method in this study was tested changing parameters, such as solvent type, the degree of derivation, wavelength range and N value and optimum parameters were chosen for this study. In this study, second order derivative UV spectrophotometric determination of glimepiride was carried out by two analysts in two different instruments with the same standard (Table 8). The result showed no statistical differences between different operators and instruments suggesting that the developed method was robust and rugged.

3.2.8. Stability

The solutions were kept in the dark at $+4$°C. Stability of glimepiride stock solutions was tested every day during 1 month and results show that glimepiride solutions in DMF were stable in this period.

The stability indicating assay was performed by stressing the glimeperide solution at a concentration of 200 μg ml^{-1} under sun light for 4 days and UV light (254 nm) for 1 day, temperature 50, 70, 100°C for 24 h and under some extreme conditions such as 0.1 N HCl and 0.1 N NaOH solutions.

When 0.1 N HCl and 0.1 N NaOH solutions were added,glimeperide was degredated while it has not been affected by other stressing affects.Degradation of glimeperide can be determined by decreasing (with 0.1 N HC), increasing (0.1 N NaOH) signal of glimeperide but degradation product(s) can not be analysed by the method presented here.

4. Conclusion

An analytical derivative UV spectrophotometric method was developed and validated thoroughly for quantitative determination of glimepiride in tablets.

The presented method was found to be rugged and robust, simple, accurate, precise, reproducible and gives an acceptable recovery of the analyte, which can be directly and easily applied to the analysis of the pharmaceutical tablet formulations of glimepiride.

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